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Cortical processing of configurally perceived odor mixtures

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INTRODUCTION

Most odors in the natural environment are not composed of a single volatile chemical species, but rather are mixtures of two to many hundreds of different volatile molecules (Thomas-Danguin et al., 2014). In some cases these mixtures depend on specific metabolic processes that result in a specific set of molecules in a specific ratio to evoke adaptive behaviors in the receiver who is especially tuned to receive that mixture (Riffell, 2012). A single component alone, even if it is the dominant component in the mixture, is insufficient to evoke the appropriate response. Thus, the mixture is perceived (i.e., drives behavior) as a synthetic configuration, distinct from its components.

In contrast to these species-specific odor mixtures, there is increasing evidence of species-non-specific configural odor processing. That is, some combinations of odorants, at a specific ratio of concentrations, are perceived configurally across a wide range of species, including insects, rodents, lagomorphs and humans (Coureaud et al, under review). For example, a 30/70 ratio of ethyl isobutyrate (a strawberry scent) and ethyl maltol (a caramel scent) is perceived as pineapple by humans – a configural percept distinct from the components. In contrast, a 68/32 ratio of the same odorants is not perceived configurally, and is not identified as pineapple scent (LeBerre et al., 2008). Data from a variety of behavioral assays, either involving explicit training or not, suggest a similar configural (or at least partially configural) perception this same 30/70 ratio of ethyl isobutyrate and ethyl maltol mixture when tested in infant rabbits (Coureaud et al., 2014), honey bees, and mice (Coureaud et al, under review). For example, mice show fear generalization between ethyl isobutyrate (A) and the elemenrtal A'B' mixture but not to the configural AB mixture (Figure 1).

Here, we took advantage of a well characterized odor set to examine single-unit and single-unit ensemble responses in the anterior piriform cortex (aPCX) and posterior PCX (pPCX) of both rats and mice to a mixture known to be perceived configurally in humans, and in a manner consistent with weak configural perception in rabbits, honey bees, and mice. We compared responses to the configural mixture with those evoked by the same chemical mixture presented at a different ratio that evokes an elemental percept, as well as to the individual chemical components.

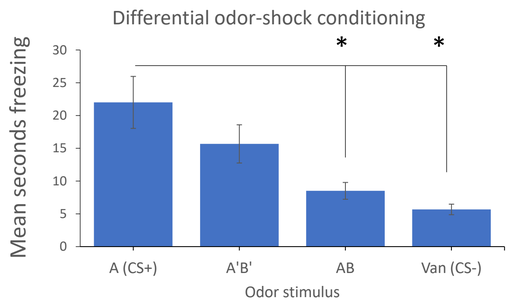


Figure 1. Generalization of odor cue-evoked freezing following differential conditioning to ethyl isobutyrate (odor A, CS+) with vanilla as CS-. During testing 24 hrs post-conditioning, mice froze significantly more to the CS+ than the CS-, and significantly more to the CS+ than the configural odor mixture AB. In contrast, freezing generalized to the elemental mixture A'B'. ANOVA, F (3, 20) = 8.146, p = 0.001. Post-hoc A vs AB or Van, p < 0.01 (signified by asterisks). Post-hoc A vs A'B', not significant.

METHODS

Animals

Long-Evans hooded rats obtained from Envigo Lab animals (200-400g) and B6SJLF1/J mice (Jackson Labs, 20-50g) were used as subjects. All procedures were approved by the Institutional Animal Care and Use Committee of the Nathan Kline Institute and were in compliance with NIH guidelines. Testing was performed during the light phase and animals has *ad lib* food and water prior to data collection.

Animals were anesthetized with urethane (1.5g/kg rats, 0.8g/kg mice) and placed in a stereotaxic apparatus. The scalp was resected and holes drilled in the skull overlying either the aPCX or pPCX. Tungsten microelectrodes (5Mohm; A-M Systems) were directed toward Layer II/III of PCX and single-unit activity recorded. Recordings were amplified (500x), band-pass filtered (0.3-3kHz), and digitized at 10kHz for data collection and analyses with Spike2 software (CED, Inc.). Local field potentials (0.3-3kHz; 200x amplification, 1kHz sample rate) were recorded simultaneously to monitor brain state during the recordings.

Once units were isolated, their basal activity rates (3 sec pre-odor onset) and response to odor (3 sec post-odor onset) were assessed. Single-units had at least 4:1 signal:noise ratio and at least 2 ms refractory period in an interval histogram. Odorant stimulation was a 2 sec pulse at 0.5 LPM directed to the nose of the freely breathing animal, with at least 30 sec between stimuli. Each stimulus was repeated three times in random order for each unit. Stimuli included ethyl isobutyrate (odor A; CAS 97-62-1; Sigma; stock solution 100.5mg in 10mL of 100% ethanol;), ethyl maltol (odor B; CAS 4940-11-8; Sigma; stock solution 100mg in 10mL of 100% ethanol), the binary mixture AB at a component ratio of 30/70 (A/B stock solutions), or the binary mixture A'B' at a component ratio of 68/32.

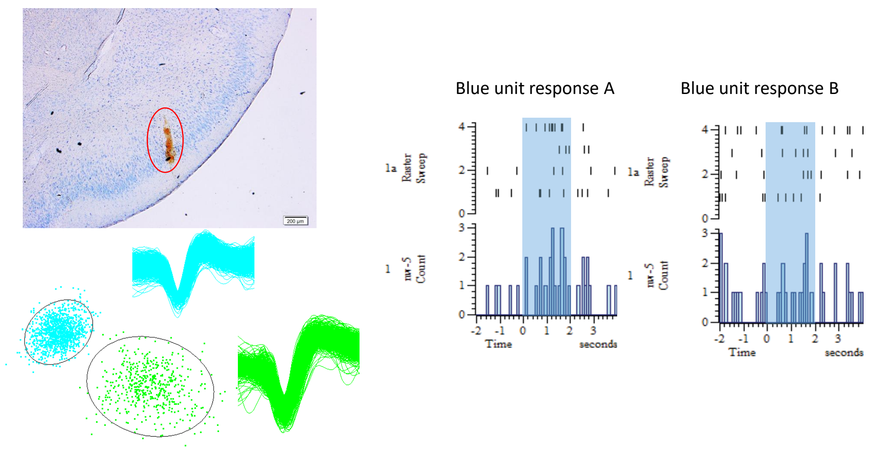


Figure 2. (**Left**) Representative histological confirmation of recordings sites with the aPCX of a rat. Two simultaneously recorded waveforms of units recorded in piriform and principle component analysis of those waveforms showing non-overlapping clusters. (**Right**) Peristimulus histograms and rasterplots of activity of those same units to odors A and B.

Histology

Following the termination of recording, animals were overdosed with urethane (3g/kg) and perfused transcardially with phosphate buffered saline and 4% paraformaldehyde. Brains were sectioned, stained with cresyl violet, and electrode placements verified with light microscopy.

Data analyses

Cumulative stimulus-evoked single-unit spike counts (number of spikes during a 3 sec period post odor onset – number of spikes during the 3 sec pre-odor onset) formed the primary dataset. Data were organized and presented as both normalized odor receptive fields and hierarchical cluster analysis (SPSS) of ensemble unit activity for each region in each species. Normalization involved expressing number of evoked spikes for a given single-unit as a proportion of the maximal response to the 'best' stimulus for that unit. The average response magnitude to a given odor was the mean of the proportional responses across cells for that odor. Thus, if all cells respond maximally (response mag. = 1.0) to EI, the mean proportional score for that odor would be 1.0.

For hierarchical cluster analyses (HCA) of how single-unit ensemble activity organized their activity to the different stimuli, standard HCA routines in SPSS were used. An agglomerative protocol was used to determine clustering and squared-euclidian distance was used to determine distance between clusters. HCA was performed for single-unit ensemble data obtained in each brain region in each species.

SUMMARY

Using a well characterized simple odor mixture that shifts between elemental and configural perceptual characteristics as component concentration ratios vary, we have identified a cross-species signature of elemental and configural coding in ensembles of PCX single-units. Future work will further clarify how this signature is expressed to other mixtures and in other species as a way more closely align odor perception with cortical odor coding.

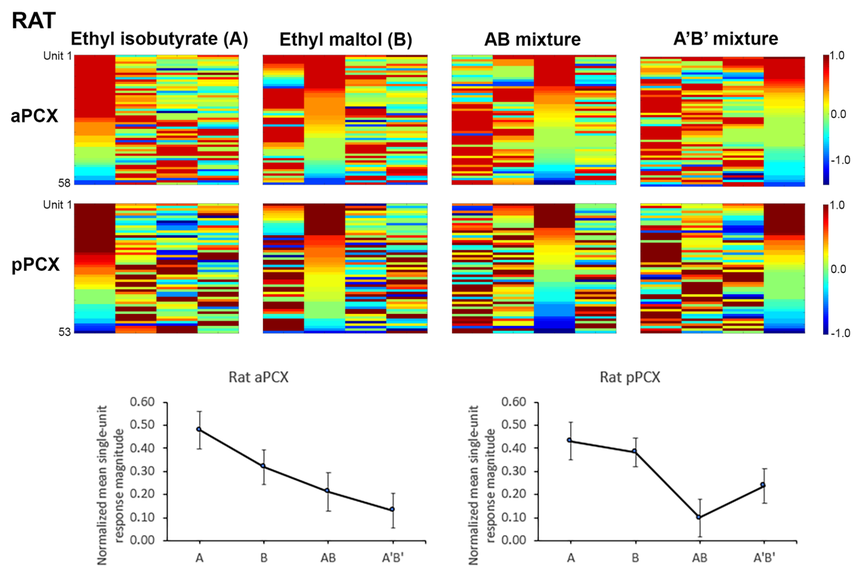


Figure 3. (**Top**) Pseudocolor plots of rat single-unit responses to two odor mixtures and their components. Each row is data from a single-unit color coded to reflect the normalized (maximal response = 1) to the four odors. The same data are replotted but sorted for cells showing their strongest response to odor A, odor B, AB or A'B'. As can be seen there are a subset of cells that are maximally responsive to each of the four different odors in both the aPCX and pPCX. (**Bottom**) Mean normalized response magnitude to each odor in the rat aPCX and pPCX. Units in both regions showed significant mixture suppression compared to their response to the components (ANOVA, $p < 0.05$).

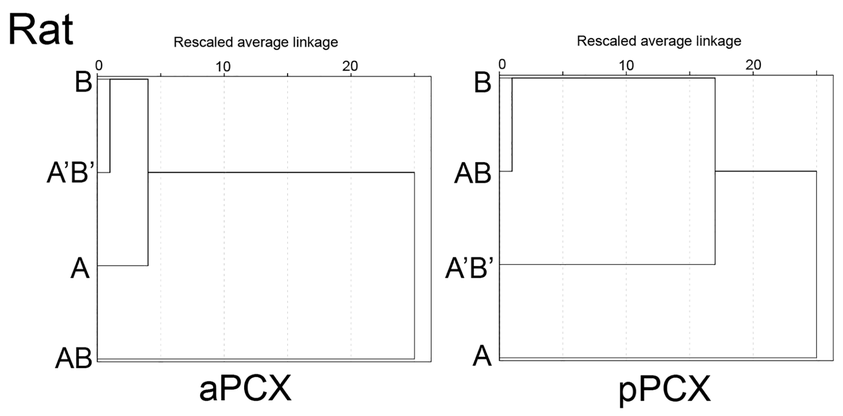


Figure 4. Hierarchical cluster analyses of rat single-unit ensembles in aPCX and pPCX to A, B, AB and A'B'. In aPCX, the component odors clustered closely with the elemental A'B' mixture, while the configural AB mixture formed its own cluster. In pPCX, while A'B' and AB occupied distinct clusters, the association with the components was less clearly organized than in aPCX.

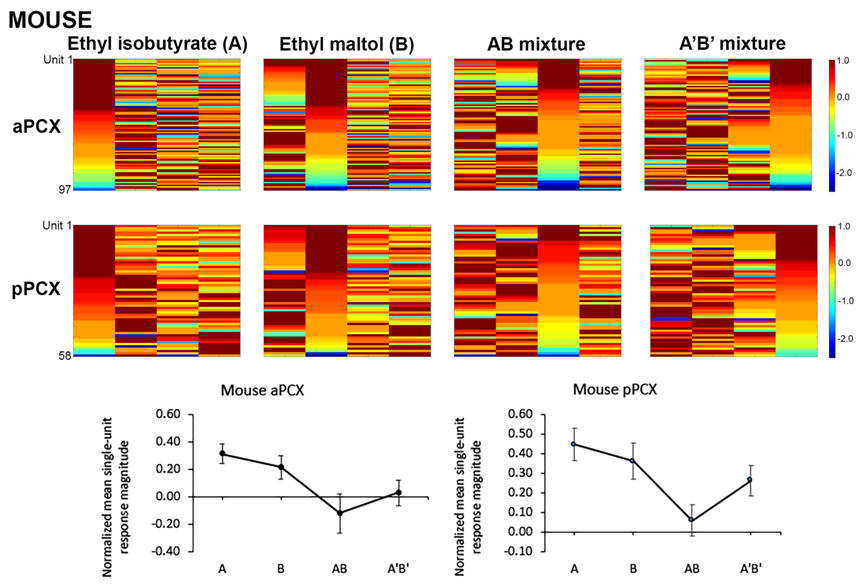


Figure 5. (**Top**) Pseudocolor plots of mouse single-unit responses to two odor mixtures and their components. As in Fig. 1, each row is data from a single-unit color coded to reflect the normalized (maximal response = 1) to the four odors, sorted for cells showing their strongest response to odor A, odor B, AB or A'B'. As can be seen there are a subset of cells that are maximally responsive to each of the four different odors in both the aPCX and pPCX. (**Bottom**) Mean normalized response magnitude to each odor in the mouse aPCX and pPCX. Units in both regions showed significant mixture suppression compared to their response to the components (ANOVA, $p < 0.05$). Note that the configural AB mixture showed the strongest mixture suppression in both aPCX and pPCX.

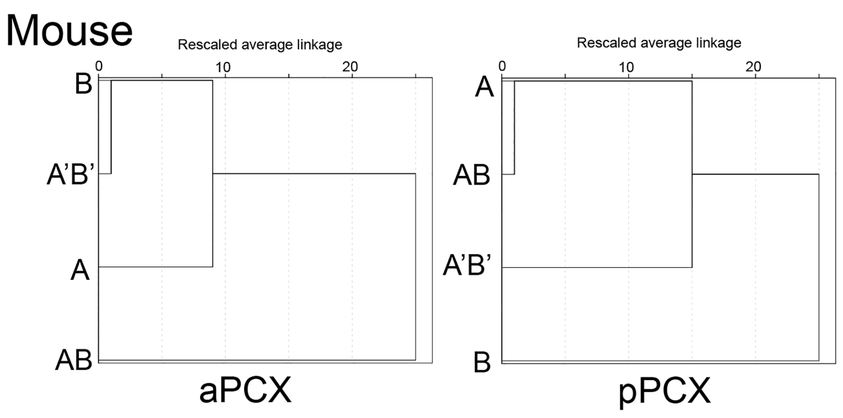


Figure 6. Hierarchical cluster analyses of mouse single-unit ensembles in aPCX and pPCX to A, B, AB and A'B'. In aPCX, the component odors clustered closely with the elemental A'B' mixture, while the configural AB mixture formed its own cluster, similar to that observed in the rat. In pPCX, while A'B' and AB occupied distinct clusters, the association with the components was less clearly organized than in aPCX.

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